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TITLE: Brain Function, Structure, and Neurochemistry after
         Tamoxifen/Chemotherapy Assessed by Neuropsychologic
         Testing and H Magnetic Resonance Spectroscopy

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Table of Contents

"Brain Function, Structure, and Neurochemistry after Tamoxifen/Chemotherapy Assess by Neuropsychologic Testing and 1H Magnetic Resonance Spectroscopy"

(1) INTRODUCTION

Loss of mental abilities represents a recognized threat to the quality of life of postmenopausal women with advancing age. Most recently, several reports have used a sensitive method (neuropsychological testing) to evaluate younger women with breast cancer after chemotherapy and hormonal modifying therapy (with tamoxifen), and found that a substantial percentage of these women had reduced mental abilities compared to women who were not treated with chemotherapy and hormonal modifying therapy. It also appears that these mental deficits are overlooked by the screening tests currently used in many large-scale breast cancer treatment and prevention studies, most likely because these simply screening tests become abnormal only when the brain is damaged to a moderate or severe degree. In the previous studies, most of the women with mental deficits obtained both chemotherapy and hormonal modifying therapy, so that it is unclear which of the two therapies caused the mental deficits. Furthermore, tens of millions of healthy women without breast cancer may soon obtain hormonal modifying therapy (with tamoxifen and possibly other drugs) to prevent future breast cancer; therefore, it is extremely important to know whether these drugs may cause injury to the brain and long-lasting problems with mental abilities. This study is designed to address these questions.

(2) BODY

Since the receipt of the funds in September 1999, we have made excellent progress towards accomplishing our goals. Altogether, we have evaluated 71 women between 65 and 80 years of age. During the first two years of the study, we made a protocol decision to focus on the three subject groups that did not involve chemotherapy, i.e. the tamoxifen, ERT and control subjects. This was done to remove and isolate Hormal effects from those associated with chemotherapy. The following paragraphs describe the research accomplished for each approved Task.

Task 1. Preparation for Subject Recruitment and Data Collection, Months 1-2

During the first 2 months of the project, we held several meetings among the key investigators and research associates to discuss and implement subject recruitment. Screening checklists were prepared, which allowed the research associates to evaluate many of the inclusion and exclusion criteria in brief telephone interviews. Because potentially millions of healthy women (without breast cancer) in the US may soon receive tamoxifen for prevention of breast cancer, we decided to focus the initial phase of the study on the evaluation of the 3 subject groups that did not receive chemotherapy, i.e. breast cancer patients on tamoxifen only (tamoxifen group); healthy women receiving estrogen replacement therapy (ERT) (positive control group), and healthy women who

did not receive ERT or tamoxifen (negative control group). This focused approach substantially accelerated our ability to answer the very important question whether tamoxifen has a negative impact on brain chemistry.

Task 2. Subject Recruitment and Data Collection, Months 3-32

During the initial project meetings, we decided to recruit women from several large ongoing studies at Harbor-UCLA Medical Center. These studies, including the WHI and WHIMS studies, involve very large cohorts of women. After obtaining approval from the local IRB and the local and overall PIs on these studies, women in the eligible age range were contacted by mail, and were asked to call a study coordinator if they were interested in participating in this study. Women who contacted the study coordinator were then asked if they would be willing to perform a brief telephone screen, which was designed to assess most of the inclusion and exclusion criteria. After passing the brief telephone screen, eligible subjects were scheduled for a visit at the Harbor-UCLA Clinical Research Center. During this visit, we first obtained verbal and written informed consent, followed by a more detailed evaluation, including routine blood tests detailed medical history, neurological examination, general functioning evaluation (Karnofsky scale), structured interviews for depressive symptoms (Geriatric Depression Scale – Short Form – 10 items, to exclude subjects with excessive depression ($\geq$ 5 years) and anxiety/panic disorder (using Form A from Phase 2 of WHIMS): yes for anxiety questions and $\geq$ 4 out of 13 questions for panic disorders). Women who did not meet the study criteria were not allowed to participate any further.

Women who did meet the inclusion and exclusion criteria were then scheduled for an MRI/MRS scan and for neuropsychological testing (on two separate occasions). Altogether, our recruitment efforts have been very successful.

Task 3. Monitor Progress of Study, Months 3-32

As specified in the proposal, the Investigators, research assistants and research associates involved with the study met on an approximately regular basis. During these meetings, we monitored the progress of the subject recruitment, and discussed and resolved problems with the study. The recruitment of new subjects was slower in year 2, for several reasons. First, a major hardware upgrade took place on the MRI scanner at the Harbor-UCLA Imaging Center. This also will make it difficult to perform direct comparisons of proton MRS data acquired prior to and after the upgrade, and we will have to scan additional healthy control subjects to establish new normative values. Second, the original PI of the proposal, Thomas Ernst, Ph.D., moved to the Brookhaven National Laboratory, NY, in the past year. We would like to transfer the role of PI to Rowan Chlebowski, M.D., Ph.D. who previously was a Co-PI (a separate request was sent to the DOD regarding this issue). Dr. Ernst would maintain responsibility for all technical aspects of the study, including data analysis.

In addition, we have focused our efforts in 2 areas: first, we have finished the neuropsychological testing in all subjects, which lagged behind during year 1 of the

study. Second, we have also performed a final analysis of the proton MRI data relating to the tamoxifen, ERT, and control groups (see next section). In the remainder of the study, we will focus on recruiting and studying elderly women who have been treated with (CMF) chemotherapy, and additional healthy control subjects (due to the scanner upgrade).

Task 4. Final Analysis and Publication, Months 33-36

As mentioned in the previous paragraph, we already have performed a final analysis of the data from the 3 study groups (tamoxifen, ERT, and control); see next paragraph.

Preliminary results

We have performed a final analysis of the ^{1}H MRS findings in women who were treated with tamoxifen, women who received ERT, and women who received neither tamoxifen or ERT. We have presented these findings at two major meetings, the 2000 American Society of Clinical Oncology (ASCO) meeting and the 2000 meeting of the International Society of Magnetic Resonance in Medicine (ISMRM); see attachments. We have also prepared a manuscript describing our findings, which has been published in the Journal of National Cancer Institute (JNCI); see attachment for a copy of the manuscript. The article was accompanied by an editorial expanding on our observations.

The manuscript reports on ^{1}H MRS in three brain regions (frontal white matter, basal ganglia, and hippocampus) in 76 elderly women, studied in three age-matched groups: 16 women receiving tamoxifen therapy, 27 women receiving estrogen replacement therapy (positive control group), and 33 women who had never received tamoxifen or estrogen (negative control group). The concentration of the putative glial marker mayo-inositol [MI] was reduced in women receiving tamoxifen or estrogen, in comparison to women in the negative control group (overall group effect on ANOVA; $p=0.02$). The [MI] in the basal ganglia showed the most pronounced decreases (-16% in the tamoxifen group and – 11% in the estrogen group), and was inversely related with the duration of tamoxifen treatment ($p=0.005$; Spearman correlation). No other significant metabolite abnormalities were observed. Because [MI] is increased in early brain injury and aging, the reduced [MI] in the tamoxifen and estrogen groups suggests that tamoxifen acts agonistic on brain estrogen receptors and has a similar neuroprotective effect as estrogen on the brain. Therefore, the findings of this study argue against our initial hypothesis that tamoxifen acts as an anti-estrogen in the brain. The lack of evidence for tamoxifen neurotoxicity on ^{1}H MRS, coupled with other accumulating evidence that tamoxifen is either non-harmful or even favorable to cognitive function, further reduce concerns about prescribing tamoxifen use for breast cancer risk reduction and other putative breast cancer risk.

(3) KEY RESEARCH ACCOMPLISHMENTS

- created infrastructure to recruit study participants from ongoing large-scale studies at Harbor-UCLA Medical Center.

- Held monthly meetings to monitor the success of patient recruitment and resolve problems.

- Recruited 71 women in the correct age range and fulfilling all exclusion and inclusion criteria.

- Finished neuropsychological testing in all subjects.

- Performed final statistical analyses of the ^{1}H MRS data for 3 groups (tamoxifen, estrogen, and negative control).

- Prepared and presented 2 abstracts at major scientific meetings.

- Presented at major breast cancer meeting

- Published an article in the Journal of the National Cancer Institute

(4) REPORTABLE OUTCOMES

Abstracts and Presentations

- Meeting of the American Society of Clinical Oncology (ASCO):
  R.T. Chlebowski, T. Ernst, L. Chang, D. Cooray, C. Salvador. Tamoxifen and Estrogen Effects on Brain Chemistry Determined by MR Spectroscopy (2001).

- Meeting of the International Society of Magnetic Resonance in Medicine (ISMRM): T. Ernst, L. Chang, K. Boone, D. Cooray, C. Salvador, R.Chlebowski. Effect of Tamoxifen Treatment on Brain Chemistry, #551 (2001).

- Workshop – Presented by R.T. Chlebowski. Cognitive Dysfunction with with adjuvant breast cancer therapy and strategies for protection – a symposium International Breast Cancer Congress, Paradise Island, Bahamas, July 31, 2002.

Publications

- T. Ernst, L. Chang, D. Cooray, C. Salvador, J. Jovicich, I. Walot, K. Boone. R. Chlebowski. Estrogen and Tamoxifen Effect on Brain Metabolism in Elderly Women: A ^{1}H MR Spectroscopy Study. J Natl Cancer Inst 94(8):592-597, 2002.

Funding

- We are in contact with Amgen, Inc., to perform a related study on the use of erythropoetin for improving CNS function.

(5) CONCLUSIONS

The concentration of the putative glial marker myo-inositol [MI] was reduced in women receiving tamoxifen or estrogen, in comparison to women in the negative control group. The concentrations of other brain metabolites, in particular N-Acetyl-aspartate (a putative neuronal marker) was normal in the ERT and tamoxifen groups. With regard to the administration of tamoxifen, this indicates that tamoxifen acts agonistic on brain estrogen receptors (i.e. is similar to estrogen), and has a similar neuroprotective effect as estrogen on the brain.

The lack of evidence to neurotoxicity on ^{1}H MRS in this study, coupled with the accumulating clinical evidence that tamoxifen is either non-harmful or even favorable to cognitive function, reduce concerns about prescribing tamoxifen use for breast cancer risk reduction and other putative breast cancer risk. This is extremely good news for women with breast cancer or those who have a high risk for developing breast cancer. It would be important to perform a larger longitudinal study with longer tamoxifen treatment periods to obtain more conclusive evidence that it is safe to utilize tamoxifen for breast cancer prevention, at least with regards to potential side effects on the brain.

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(7) APPENDICES

1. Abstract Meeting of the American Society of Clinical Oncology (ASCO): R.T. Chlebowski, T. Ernst, L. Chang, D. Cooray, C. Salvador, Tamoxifen and Estrogen Effect on Brain Chemistry Determined by MR Spectroscopy (2001).

2. Abstract Meeting of the International Society of Magnetic Resonance in Medicine (ISMRM): T. Ernst, L. Chang, K. Boone, D. Cooray, C. Salvador, R. Chlebowski. Effect of Tamoxifen Treatment on Brain Chemistry, #551 (2001).

3. Ernst T, Chang L, Boone K, Cooray D, Salvador C, Chlebowski RT. Tamoxifen and estrogen effects on brain chemistry determined by MR Spectroscopy. J Natl Cancer Inst 94(8): 592-597, 2002.

4. R. Chlebowski. Estrogen and Tamoxifen – Are they neuroprotective? presentation from the Cognitive Dysfunction with Adjuvant Breast Cancer Therapy and Strategies for Protection workshop at the International Breast Cancer Congress, Paradise Island, Bahamas, July 31, 2002.

TAMOXIFEN AND ESTROGEN EFFECTS ON BRAIN CHEMISTRY DETERMINED BY MR. SPECTROSCOPY

R.T. Chlebowski, Harbor-UCLA Research and Education Institute, Torrance, CA, T. Ernst, L. Chang, Brookhaven National Laboratory, Upton, NY, D. Cooray, C. Salvador, Harbor-UCLA Research and Education Institute, Torrance, CA

Observational studies suggest cognitive function may be under hormonal influence. Since tamoxifen use for risk reduction can be considered in otherwise healthy women, we explored the effects of tamoxifen and estrogen on brain chemistry in elderly ($\geq$65 years) women using localized ^{1}H magnetic resonance spectroscopy (MRS). MRI and localized ^{1}H MRS were performed in 76 women: 16 breast cancer patients (age 69.8±4.7 years) treated with tamoxifen (mean treatment period 4.4±1.7 years) who did not receive chemotherapy, 27 healthy women (age 71.4±4.0 years) treated with estrogen replacement therapy (ERT) mean treatment period 20.8±10.5 years, and 33 healthy women (age 71.7±4.5 years) who never took tamoxifen or estrogen. The cerebral metabolite concentrations of N-Acetyl compounds [NA], total creatine [CR], total choline [CHO] and myo-inositol [MI] were determined in the frontal white matter, basal ganglia, and hippocampus, using an established protocol for absolute quantitation. As seen below, the tamoxifen group had significantly lower [MI] in the basal ganglia (-13%; $p<0.05$) compared to controls who never received hormonal treatment; [MI] in the basal ganglia was negatively correlated with the tamoxifen treatment period ($p=0.005$; Spearman correlation coefficient = -0.72). Although there was a trend ($p=0.12$) for lower MI in the basal ganglia in the ERT group, no other significant metabolite differences were observed between the three groups in any of the 3 brain regions evaluated. Since normal aging and some cognitive dysfunctional status are associated with increase in the glial marker [MI], the reduction in [MI] in women receiving tamoxifen suggests tamoxifen may be associated with favorable modulation of regional "brain aging". To test this hypothesis, ongoing neuropsychological data will relate the ^{1}H MRS findings to clinical cognitive function.

Cerebral Metabolites (mmoles/kg) in Basal Ganglia Of Women by Treatment Group

Group	[NA]	[CR}	[CHO}	[MI]
Tamox.	8.5± 0.2	8.9± 0.3	2.1± 0.1	6.3± 0.3
Control	8.7± 0.2	8.9± 0.2	2.1±0.1	7.3± 0.3
ERT	8.8± 0.2	9.0± 0.2	2.1± 0.1	6.7± 0.3
*significance p<0.05 versus control				

Effect of Tamoxifen Treatment on Brain Chemistry

Thomas Ernst, Linda Chang, Kyle Boone, Dilrukshie Cooray, Corazon Salvador, Rowan Chlebowski Brookhaven National Laboratory, NY, and Harbor-UCLA Medical Center, Torrance, CA

Background: A substantial percentage of women with breast cancer after chemotherapy and hormonal modifying therapy (with tamoxifen) have reduced mental abilities compared to women who were not treated with chemotherapy and tamoxifen (1). Because tens of millions of healthy women who are at risk for breast cancer may soon obtain the anti-estrogen tamoxifen (and possibly other drugs) for preventative therapy, it is extremely important to know whether these drugs may cause injury to the brain (2).

Objective: To evaluate the potential effects of tamoxifen on brain chemistry, using localized ^{1}H magnetic resonance spectroscopy (MRS).

Design and Methods: MRI and localized ^{1}H MRS were performed in 16 women with breast cancer who were treated with tamoxifen (mean age 69.8±4.7 years; mean tamoxifen treatment period 4.4±1.7 years), in 27 healthy women who have been treated with estrogen replacement therapy (ERT) (mean age 71.4±4.0 years; average treatment period 20.8±10.5 years), and in 33 healthy women (mean age 71.7±4.5 years) who were never treated with tamoxifen or estrogen. The cerebral metabolite concentrations of N-Acetyl compounds [NA], total creatinine [CR], total choline [CHO] and myo-inositol [MI], were determined in the frontal white matter, basal ganglia, and hippocampus, using a protocol for absolute quantitation (3, 4).

Table: Cerebral metabolite concentrations (in mmoles/kg). Significance: * : p < 0.05

|		[NA]	[CR]	[CHO]	[MI]
Basal ganglia	Tamox.	8.51 ±0.18	8.92 ±0.29	2.06 ±0.07	6.33 ±0.28*
	Control	8.66 ±0.17	8.92 ±0.18	2.14 ±0.06	7.27 ±0.28
	ERT	8.75 ±0.15	8.97 ±0.19	2.13 ±0.07	6.65 ±0.31
Frontal WM	Tamox.	7.39 ±0.17	6.69 ±0.20	1.69 ±0.05	7.5 1±0.30
	Control	7.42 ±0.13	6.52 ±0.14	1.76 ±0.05	7.50 ±0.19
	ERT	7.49 ±0.14	6.37 ±0.13	1.67 ±0.06	7.19 ±0.23
Hippo-campus	Tamox.	9.04 ±0.24	8.66 ±0.27	2.80 ±0.09	10.1 ±0.32
	Control	8.62 ±0.17	8.32 ±0.18	2.79 ±0.07	10.28 ±0.32
	ERT	8.69 ±0.21	8.51 ±0.19	2.74 ±0.08	9.74 ±0.28
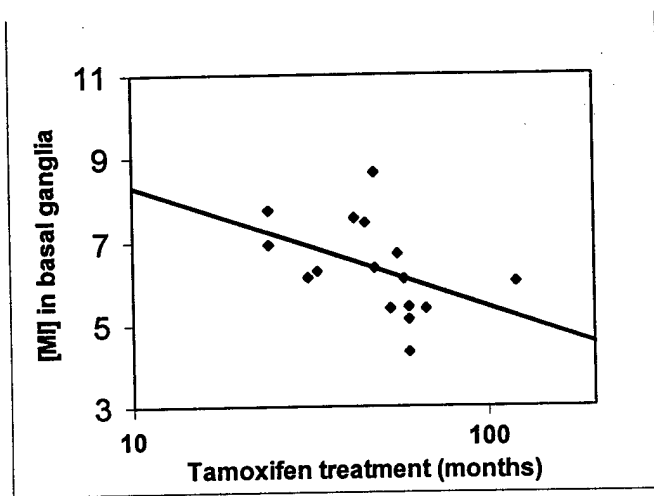
Figure: Relationship between the [MI] in the basal ganglia and the number of months patients were treated with tamoxifen.

Results: The tamoxifen group had lower [MI] in the basal ganglia (-13%; p<0.05) compared to women who were never treated with tamoxifen (see Table). The [MI] in the basal ganglia was negatively correlated with the tamoxifen treatment period (p=0.005; Spearman correlation coefficient = -0.72; see Figure). No other metabolite differences were observed between the two groups in any of the 3 brain regions evaluated.

Discussion: Our preliminary data indicate that women who have been using tamoxifen have reduced [MI] in the basal ganglia compared to age-matched women who did not receive tamoxifen. Since normal aging has been shown to be associated with increases in the glial marker [MI] (5), the reduced value in women on tamoxifen may be interpreted (with caution) as "slowing of brain aging", at least in the basal ganglia region. Alternatively, reduced [MI] may indicate abnormalities in the osmotic state of brain tissue (6). Future analyses of neuropsychological data will relate the ^{1}H MRS findings to cognitive function.

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Estrogen and Tamoxifen
- Are they Neuroprotective?

Rowan T. Chlebowski, M.D., Ph.D.

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UCLA School of Medicine
Research and Education Institute at
Harbor-UCLA Medical Center

Hormone Therapy and Cognition
Systemic Review and Meta-Analysis

Randomized controlled trials and cohort
studies for cognitive decline

Cohort and case-control studies for
dementia risk

29 studies identified, cognition studies could
not be quantitatively combined

LeBlanc, JAMA 285:1489, 2001

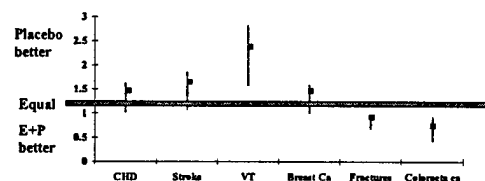## Hormone Therapy and Cognition
Systemic Review and Meta-Analysis

Women with menopausal symptoms:
improved verbal memory, vigilance,
reasoning, motor speed

Asymptomatic women: no benefit

Hormones and dementia: OR 0.66 (0.53-0.82)

LeBlanc, JAMA 285:1489, 2001

Relative Risks of Events of E+P
Compared to Placebo

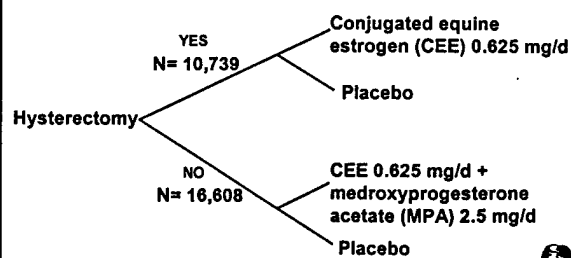---

Risks and benefits of estrogen plus progestin in healthy postmenopausal women

Principal results of the Women's Health
Initiative randomized controlled trial
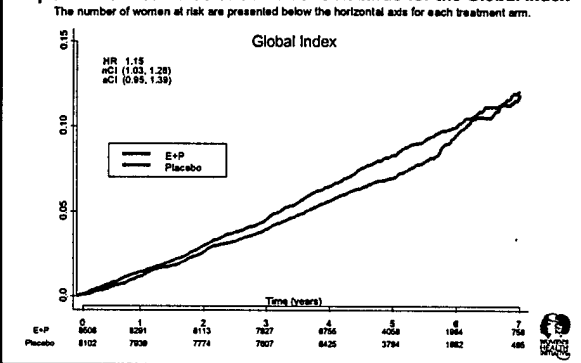
WOMEN'S
HEALTH
INITIATIVE

WHI Hormone Program Design

Hysterectomy

YES
N= 10,739

Conjugated equine
estrogen (CEE) 0.625 mg/d

Placebo

NO
N= 16,608

CEE 0.625 mg/d +
medroxyprogesterone
acetate (MPA) 2.5 mg/d

Placebo

Women's Health Initiative E plus P Effects

Unfavorable	Favorable
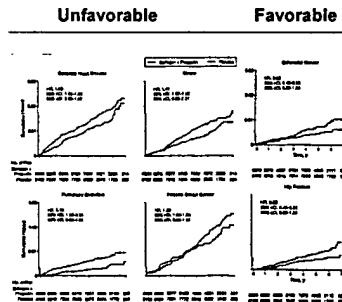
Kaplan-Meier Estimates of Cumulative Hazards for the Global Index

The number of women at risk are presented below the horizontal axis for each treatment arm.

If a long duration of estrogen or estrogen plus progestin use is needed for cognitive effect, will toxicity profile (of at least E + P) allow women to "get there"?

Women's Health Initiative Memory Study (WHIMS)
Ancillary Study to WHI in Women ≥65 yrs

Double-blind, randomized, placebo controlled long term stop of E plus P and E alone

8,300 women entered

Modified mini-mental state (3 MS) if positive on screen - neuropsychological testing and evaluation

80% power for 40% reduction in rate of all-cause dementia with hormones

Shumaker, Control Clin Trial 19:604, 1998

WHIMS stopped early as WHI identified "more risk than benefit" based on other clinical endpoints

Available data on mini-mental state under analysis and has been requested by FDA for review

Note power considerations: Mean F/U of 5.2 yrs vs design plans for 8.24 yrs F/U implications for other trials

JAMA 288:321, 2002

Hormones and Cognition
Large-Scale Long Term Trials

WHI

WHISCA

WHIMS

WISDOM-COG

PREPARE

Zec Climacteric 5:122, 2002

What about other approaches to this issue?

Rationale and Hypothesis

- Estrogen: suggested positive effects on brain chemistry and function
- Tamoxifen: increased hot flashes; ? Estrogen antagonist in CNS
- Assuming brain injury / degeneration $\Rightarrow \Uparrow$ [MI] (glial activation)
- Hypothesis (predicted results):
  - Tamoxifen: increased [MI] (neurotoxic)
  - Estrogen: decreased [MI] (neuroprotective)

Study Design

- Cross Sectional Observational Study
- Eligibility:
  - Women ages 65-80 years
  - Fluent in English
  - Conforming to one of three groups:
    - Tamoxifen group: Women with diagnosed breast cancer receiving tamoxifen (20 mg/day) for > 2 years and < 5 years. No prior systemic chemotherapy or hormone replacement therapy (HRT).
    - Positive control group: Healthy women, matched to tamoxifen group by age and education, receiving HRT with estrogen for > 2 years.
    - Negative control group: Healthy women, matched to tamoxifen group by age and education, no prior tamoxifen, chemotherapy, or estrogen.
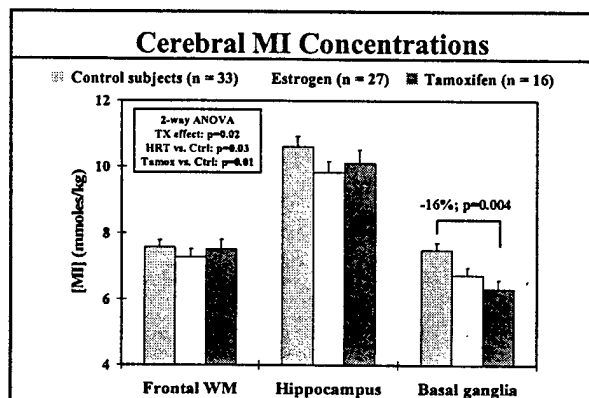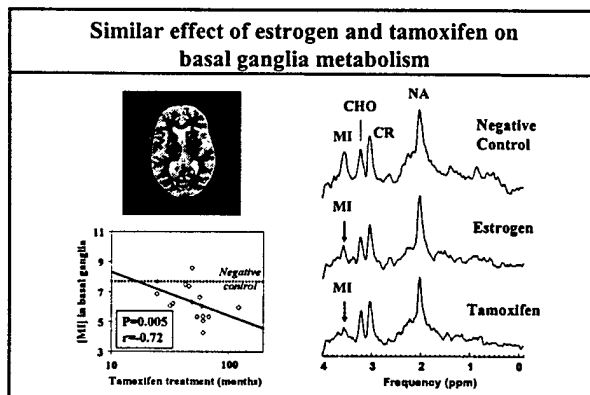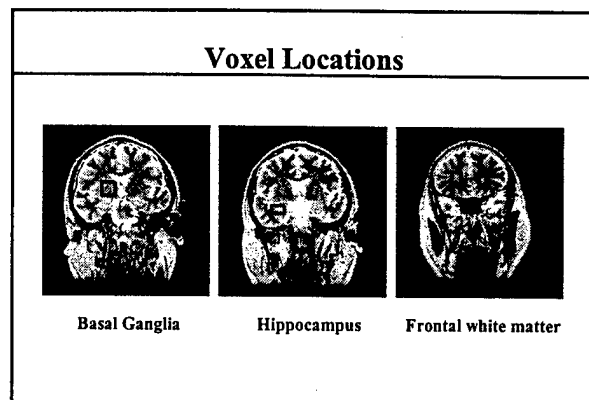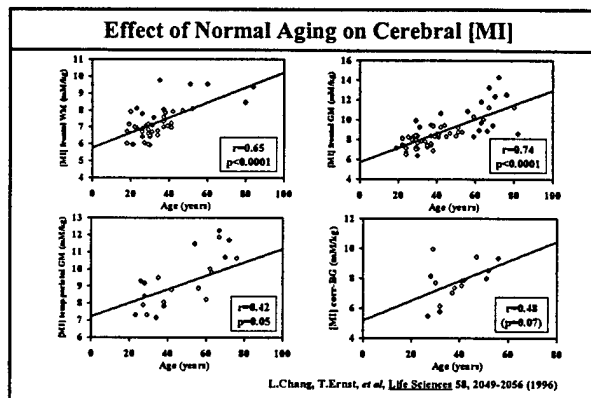
Exclusion Criteria

Psychiatric disorders depression requiring treatment; significantly depressed or having anxiety/panic disorder on screening, schizophrenia; drug dependence [including alcohol]; pacemaker; metallic objects in head or body; uncontrolled hypertension, diagnosis of: diabetes, Alzheimer disease, or Parkinson's disease; history of head trauma with loss of consciousness for more than 1 hour; recurrent breast cancer (in tamoxifen group).

^{1}H Magnetic Resonance Spectroscopy

- Non-invasive in vivo measurement of structural neurochchemicals within a localized brain region.
- Myo-Inosital [MI] (glial marker)
- N-acetyl-aspartate [NA] (neuronal marker)
- Cohline - containing compounds [CHO] (cell membrane marker)
- Creatine plus phosphocreatine [CR] (energy metabolism marker)

Group Participant Characteristics

	Tamoxifen	HRT	Control
number	16	27	33
Age (mean)	68.9 ± 4.7	71.2 ± 4.1	71.6 ± 4.7
Tamoxifen Use (yrs) (20 mg/d)	4.4 ± 1.7	0	0
HRT Use (yrs) (Premarin 0.625 mg/d)	0	20.8 ± 10.5	0
Hypertension (controlled) (average yrs since diagnosis)	3.5	4.1	3.1
Cholesterol (mg/dl)	233	211	222

Effect of Normal Aging on Cerebral [MI]

L.Chang, T.Ernst, et al, Life Sciences 58, 2049-2056 (1996)

Voxel Locations

Basal Ganglia   Hippocampus   Frontal white matter

Similar effect of estrogen and tamoxifen on basal ganglia metabolism

P=0.005
r=-0.72

CHO
NA
MI | CR   Negative Control
MI   Estrogen
MI   Tamoxifen

Cerebral MI Concentrations

Control subjects (n = 33)   Estrogen (n = 27)   Tamoxifen (n = 16)

2-way ANOVA
TX effect: p=0.02
HRT vs. Ctrl: p=0.03
Tamox vs. Ctrl: p=0.01

-16%; p=0.004

Frontal WM   Hippocampus   Basal ganglia

Conclusions

→ Tamoxifen
- Has similar effect on ^{1}H MRS as estrogen ⇒ estrogen agonist?
- Favorable modulation of "brain aging"
  - 1.2mM decreased [MI] corresponds to 15-20 years of reduced aging
- Supportive evidence:
  - 1,385 nursing home women – Tamoxifen use was associated with significantly reduced frequency of Alzheimer's Disease, better ADLs & cognitive function (Breuer B, Anderson R. Women Health 2000;31:71-85)
- These results predict tamoxifen may have neuroprotective effects

Cognitive Function on Tamoxifen in Breast CA Pts

Recruited from prior case control study

1,163 women 57 75yrs old (710 tamoxifen)
Cognitive Function: clock drawing, copying a box drawing, narrative writing

No differences on standard tests

By history women with 5 yrs tamoxifen saw MD for "memory problems" (3.8 vs 1.5%, p = 0.04)

Current users had lower complexity score (p = 0.03)

Paganini-Hill, Breast Ca Res Treat 64:165, 2000

Can Observational Studies Provide Reliable
Information on Effects of Estrogen and/or
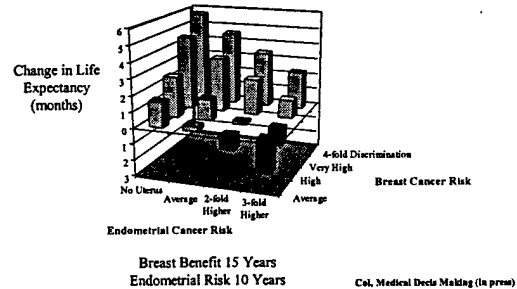Progestin on Cognition/Dementia?

Note:

Observational Studies predicted 30%
reduction in CHD with E + P

WHI randomized trial found 29%
increase in CHD with E + P

Tamoxifen Impact on Survival:
Analysis Based On Additional "Carry Over" Effect

Change in Life
Expectancy
(months)

4-fold Discrimination
Very High
High   Breast Cancer Risk
No Uterus
Average 2-fold 3-fold Average
Higher Higher
Endometrial Cancer Risk

Breast Benefit 15 Years
Endometrial Risk 10 Years   Col, Medical Decis Making (In press)

JAMA Website

http://www. jama.ama-assn.org/

Summary

- Decreased [MI] with both tamoxifen and estrogen;
  most pronounced in basal ganglia
- No changes in other metabolites

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Tamoxifen and Estrogen Effects on Brain Chemistry Determined by MRI Spectroscopy.

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Abstract No: 108

Category: Adjuvant Therapy

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Abstract: Observational studies suggest cognitive function may be under hormonal influence. Since tamoxifen use for risk reduction can be considered in otherwise healthy women, we explored the effects of tamoxifen and estrogen on brain chemistry in elderly ($\geq$65 years) women using localized ^{1}H magnetic resonance spectroscopy (MRS). MRI and localized ^{1}H MRS were performed in 76 women: 16 breast cancer patients (age 69.8?4.7 years) treated with tamoxifen (mean treatment period 4.4?1.7 years) who did not receive chemotherapy, 27 healthy women (age 71.4?4.0 years) treated with estrogen replacement therapy (ERT) mean treatment period 20.8?10.5 years, and 33 healthy women (age 71.7?4.5 years) who never took tamoxifen or estrogen. The cerebral metabolite concentrations of N-Acetyl compounds [NA], total creatine [CR], total choline [CHO] and myo-inositol [MI] were determined in the frontal white matter, basal ganglia, and hippocampus, using an established protocol for absolute quantitation. As seen below, the tamoxifen group had significantly lower [MI] in the basal ganglia (-13%; p<0.05) compared to controls who never received hormonal treatment; [MI] in the basal ganglia was negatively correlated with the tamoxifen treatment period (p=0.005; Spearman correlation coefficient = -0.72). Although there was a trend (p=0.12) for lower MI in the basal ganglia in the ERT group, no other significant metabolite differences were observed between the three groups in any of the 3 brain regions evaluated. Since normal aging and some cognitive dysfunctional status are associated with increase in the glial marker [MI], the reduction in [MI] in women receiving tamoxifen suggests tamoxifen may be associated with favorable modulation of regional "brain aging". To test this hypothesis, ongoing neuropsychological data will relate the ^{1}H MRS findings to clinical cognitive function.

Cerebral Metabolites (mmoles/kg) in Basal Ganglia of Women by Treatment Group

Group	[NA]	[CR]	[CHO]	[MI]
Tamox.	8.5?0.2	8.9?0.3	2.1?0.1	6.3?0.3*
Control	8.7?0.2	8.9?0.2	2.1?0.1	7.3?0.3
ERT	8.8?0.2	9.0?0.2	2.1?0.1	6.7?0.3
*Significance p<0.05 versus control				

http://www.asco.org/asco/publications/abstract_print_view/0,1148,_12-002326-00_29-00...   12/5/2002

The Effects of Tamoxifen and Estrogen on Brain Metabolism in Elderly Women

Thomas Ernst, Linda Chang, Dilrukshie Cooray, Corazon Salvador, Jorge Jovicich, Irwin Walot, Kyle Boone, Rowan Chlebowski

Background: Tamoxifen is used to treat breast cancer and may reduce the risk of breast cancer. However, there are conflicting reports as to whether tamoxifen use is associated with changes in brain metabolism and function or cognitive impairment. Consequently, we assessed the effects of tamoxifen and estrogen on the brain chemistry of elderly women. *Methods:* We used proton magnetic resonance spectroscopy to measure the concentrations of N-acetyl-containing compounds, *myo*-inositol (MI), total creatine (creatine plus phosphocreatine), and choline-containing compounds in the frontal white matter, basal ganglia, and hippocampus of 76 elderly women of whom 16 had received tamoxifen therapy, 27 had received estrogen as hormone replacement therapy (HRT), and 33 had received neither (control group). A two-way analysis of variance (ANOVA) was performed to determine the statistical significance of differences in cerebral metabolite concentrations among subject groups and brain regions. All statistical tests were two-sided. *Results:* Women in the tamoxifen and HRT groups had lower concentrations of MI in all areas than women in the control group ($P = .02$; overall group effect on ANOVA). Compared with the control group, the tamoxifen group ($P = .004$) and the HRT group ($P = .06$) had lower concentrations of MI in their basal ganglia. The MI concentration in the basal ganglia was inversely correlated with the duration of tamoxifen treatment ($\rho = -.72$; $P = .005$). *Conclusions:* The reduced concentrations of MI in the brains of women treated with tamoxifen and HRT, compared with those of control women, suggest that tamoxifen has an effect similar to that of estrogen. These results, if confirmed, may alleviate concerns about the safety of using tamoxifen to reduce breast cancer risk in elderly women. [J Natl Cancer Inst 2002;94:592–7]

Tamoxifen is an estrogen receptor agonist and antagonist that is widely used to treat breast cancer *(1)*. Recent results suggest that tamoxifen also reduces the risk of developing breast cancer *(2,3)* and may be offered for this indication after the long-term risks and benefits are considered *(4–7)*. Two studies *(8,9)* have demonstrated that estrogen has positive effects on brain metabolism and function. However, a preclinical study *(10)* has suggested that tamoxifen may also act as an estrogen antagonist in the brain. In addition, the combined use of tamoxifen and chemotherapy in the clinic has been associated with cognitive impairment *(11)*. Such results have led to the hypothesis that tamoxifen may negatively affect brain metabolism, especially in elderly women.

We used proton magnetic resonance spectroscopy (^{1}H MRS), a neuroimaging technique that can measure the concentrations of biochemical markers associated with brain injury, to perform a cross-sectional study to compare brain metabolism in women with breast cancer who had received tamoxifen with that in healthy women who had received hormone replacement therapy

(HRT) without tamoxifen and with that in healthy women who had not received either tamoxifen or HRT. We determined the concentrations of four metabolites: N-acetyl-containing compounds (NA), including N-acetyl-L-aspartate, a neuronal marker that reflects neuronal density and integrity *(12)*; *myo*-inositol (MI), a putative glial marker whose levels reflect glial content or activity *(13)*; total creatine (CR), which reflects high-energy phosphate metabolism *(14)*; and choline-containing compounds (CHO) associated with cell membrane metabolism *(15)*. Numerous MRS studies [e.g., *(16,17)*] in patients with various neurologic disorders have suggested an association between increased cerebral MI and glial proliferation in response to brain injury. Therefore, we hypothesized that an estrogen-antagonistic effect of tamoxifen might be associated with increased cerebral MI concentrations, indicating brain injury.

SUBJECTS AND METHODS

Study Subjects

Three groups of age-matched women (total n = 76) between the ages of 65 and 80 years were recruited from the local community, a suburban area in the southwestern part of Los Angeles County, through advertisements, and from solicitations through the mail. Recruitment in all three subject groups occurred in parallel, and women fulfilling the study criteria were enrolled immediately. Verbal and written informed consent were obtained from each woman, according to procedures approved by the Institutional Review Board of the Harbor-UCLA Research and Education Institute (REI) and in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. The tamoxifen group (n = 16) included women who were diagnosed with localized breast cancer, had undergone resection, and had received tamoxifen (20 mg/day) for at least 2 years (range = 2–10 years; mean ± standard deviation (SD) = 4.4 ± 1.7 years) but had never received any systemic chemotherapy or estrogen as HRT. The HRT group (n = 27) consisted of healthy women with no history of breast cancer who had received HRT for at least 2 years (range = 2–55 years; mean ± SD = 20.8 ± 10.5 years). HRT consisted of oral conjugated equine estrogen (Premarin; Wyeth, Madison, NJ) at 0.625 mg/day [n = 25]; Premarin at 1.25 mg/day [n = 1]; and estropipate at 0.75 mg/day [n = 1]); in addition, three women

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See "Notes" following "References."

who received Premarin at 0.625 mg/day also received intermittent medroxyprogesterone (Provera; Pharmacia Upjohn Co., Kalamazoo, MI) at 10 mg/day. The control group (n = 33) consisted of healthy women with no history of breast cancer who were never treated with tamoxifen, chemotherapy, or HRT. All study subjects were evaluated by a physician at the General Clinical Research Center at Harbor-UCLA REI to ensure that they did not have recurrent breast cancer, psychiatric disorders, chronic medical or neurologic illnesses (e.g., uncontrolled hypertension, abnormal thyroid function, diabetes, strokes, Alzheimer's disease, or Parkinson's disease) that might affect cognition, a history of head trauma with loss of consciousness for more than 1 hour, or any contraindication for the imaging studies, any of which would have excluded them from our study. Clinical evaluations of the study subjects included a medical history, physical and neurologic examination, blood pressure assessments, an electrocardiogram, a battery of comprehensive screening blood tests (complete blood count, chemistry panel, thyroid function tests, and quantitation of rapid plasma reagin, vitamin B_{12}, and folate levels), and urinalysis. Each subject also completed a standardized cognitive screening form [modified Mini-Mental State Examination (18)] and two neuropsychologic tests, the Digit Symbol Substitution Test and the Trail Making Test-part A (19), which evaluated psychomotor speed.

Imaging Studies

Magnetic resonance imaging (MRI) scans were performed on a 1.5-Tesla scanner (General Electric Medical Systems, Milwaukee, WI). After obtaining a sagittal T1-weighted localizer (echo time [TE]/relaxation time [TR] = 11/500 ms, 5-mm thickness, 1-mm gap), we performed an axial fast spin echo sequence (TE1/TE2/TR = 17/102/4000 ms, 5-mm thickness, no gap) and an axial fluid-attenuated inversion recovery sequence (TE/inversion time [TI]/TR = 142/2600/11000 ms) on each study subject. We then performed localized ^{1}H MRS in each of three volumes of interest in the brain: the frontal white matter, the basal ganglia, and the left hippocampal region. The volumes of interest ranged between 3 and 5 cm^3. We were very careful to ensure that each volume of interest included the same anatomic structures across

subjects. The volume of interest in the basal ganglia contained mostly deep gray matter (putamen, globus pallidus, and a portion of the head of the caudate) and a small portion of the white matter (internal capsule) (Fig. 1). MRS data for each volume of interest were acquired using a double spin echo sequence, which was optimized for the acquisition of ^{1}H MR spectra from the frontal lobe (20,21). The acquisition parameters for all volumes of interest were TE/TR = 30/3000 ms, and 64 traces were averaged. We determined the cerebral concentrations of NA, MI, CR, and CHO, corrected for the presence of cerebrospinal fluid in each voxel, as previously described (22). Typical intrasubject variabilities in the concentrations of NA, CR, CHO, and MI were less than 10% (23).

Statistical Analyses

Statistical analyses were performed in StatView version 5.0 (SAS Institute, Cary, NC). A mixed two-way analysis of variance (ANOVA) was performed to determine the statistical significance of differences in cerebral metabolite concentrations among the three brain regions (within-variable; random effect) and among the three subject groups (between-variable; fixed effect). Kolmogorov–Smirnov tests demonstrated that the cerebral metabolite concentrations were normally distributed. A type I error probability of $P \leq .05$ was used to determine statistical significance for main effects on the ANOVA. For variables that showed statistical significance on ANOVA, Student's t tests were performed to determine the statistical significance of differences between individual groups for each brain region. Because nine of these t tests were performed for each variable (three groups times three brain regions), only differences with a probability $P < .0055$ ($= .05/9$) were considered statistically significant for the post hoc tests. Spearman correlations were performed to test for possible relationships between metabolite concentrations and the duration of treatment with tamoxifen or estrogen. A probability of $P \leq .05$ was used to determine statistical significance for these correlation analyses. All statistical tests were two-sided.

Fig. 1. Representative proton magnetic resonance spectra from the basal ganglia region of a subject from each of the three treatment groups (right). The area under each peak was used to determine metabolite concentrations. The concentration of *myo*-inositol (MI) was lower in the woman who received hormone replacement therapy (HRT) or tamoxifen than in the woman who received neither (Control). The voxel location in the basal ganglia is indicated in the axial magnetic resonance imaging (MRI) (top left). The graph (bottom left) shows the dependence of the MI concentration (in mmol/kg) in the basal ganglia on the duration of tamoxifen treatment (logarithmic scale). NA = N-acetyl resonance; CR = total creatine (creatine plus phosphocreatine) resonance; CHO = resonance of choline-containing compounds; ppm = parts per million.
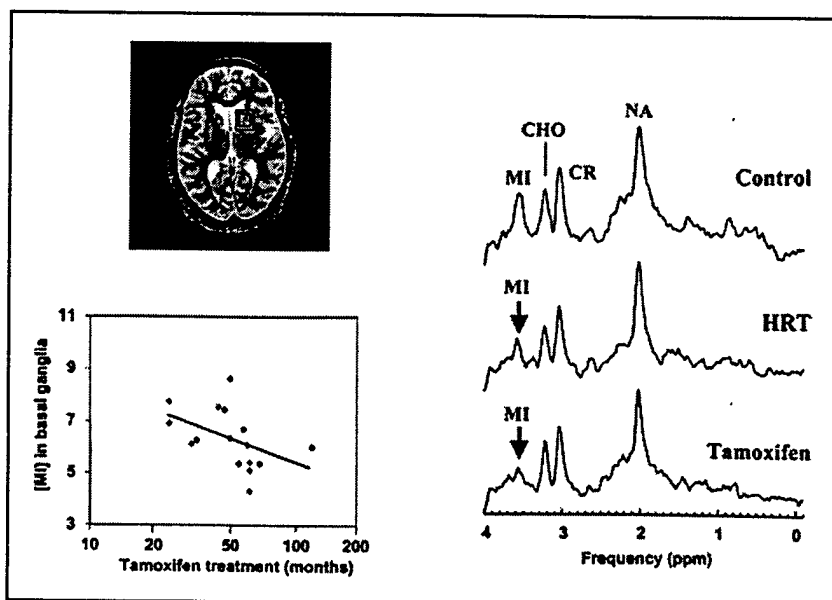
RESULTS

The ages of the women in the three groups were tightly matched and not statistically significantly different among the groups (P = .52). The mean (± SD) ages were 71.8 (± 4.1) years for the control group, 71.5 (± 4.1) years for the HRT group, and 70.4 (± 4.7) years for the tamoxifen group. There was no statistically significant difference in the average number of years of education among the three groups (control group = 14.1 years; HRT group = 14.8 years; tamoxifen group = 13.2 years; P = .15). We detected no cognitive deficits in any of the women or intergroup differences in cognition; for example, on a scale of 0–100 for cognitive assessment (with 100 being the highest cognitive score), women in the tamoxifen, HRT, and control groups had mean (± SD) cognitive scores of 95.9 (± 3.5), 95.4 (± 4.5), and 95.2 (± 4.9), respectively. The only finding upon neurologic examination was mild essential tremor in 19 women (six women [22%] in the HRT group, five women [31%] in the tamoxifen group, and eight women [24%] in the control group). The results of two neuropsychologic tests that are sensitive to psychomotor speed and that can detect motor disorders in the upper extremities were not statistically significantly different among the three groups. For example, in the Digit Symbol Substitution Test, the number of correct substitutions after 90 seconds (± SD) for women in the HRT, tamoxifen, and control groups were 7.0 ± 1.7, 7.5 ± 3.1, and 7.2 ± 2.1 substitutions, respectively (P = .91), whereas in the Trail Making Test-part A, the time (± SD) required to connect 20 consecutively numbered circles was 38.8 ± 13.8 seconds for women in the HRT group, 44.2 ± 12.2 seconds for women in the tamoxifen group, and 36.9 ± 10.4 seconds for women in the control group (P = .27). Structural MRI scans showed no major structural abnormalities (i.e., cortical infarcts, tumors, or vascular malformations) in any study subject. However, nearly half of all the women (49%) had small or moderate white matter hyperintensities in the periventricular regions; no intergroup differences were observed with respect to these lesions. We also observed possible silent lacunar infarcts (small lesions showing hyperintensity on T2-weighted MRI and hypointensity on T1-weighted images) in three women in the HRT group but not in any women in the other two groups.

The ANOVA demonstrated a statistically significant difference in the MI concentrations among the three treatment groups (P = .02; group effect on ANOVA) and among the three brain regions (P<.001; brain region effect on ANOVA). However, MI concentration did not differ statistically significantly among the three brain regions by treatment group (P = .50; interaction between treatment group status and brain region). The overall concentration of MI was statistically significantly lower in the tamoxifen (P = .01) and HRT (P = .03) groups than in the control group. The difference in the MI concentration among treatment groups was most pronounced in the basal ganglia. For example, the MI concentration in the basal ganglia was statistically significantly lower in the tamoxifen group (6.33 mmol/kg) than it was in the control group (7.57 mmol/kg) (P = .004; difference in MI concentration between the tamoxifen group and the control group = –1.24 mmol/kg [95% confidence interval {CI} = –0.43 to –2.05 mmol/kg]; Table 1). The MI concentration was also lower in the basal ganglia of the HRT group (6.73 mmol/kg) than it was in the control group (7.57 mmol/kg), but that difference was not statistically significant (P = .06; difference in MI concentration between HRT group and control group = –0.83 mmol/kg [95% CI = –1.71 to 0.05 mmol/kg]). There was an inverse relationship between the duration of tamoxifen treatment and the MI concentrations in the basal ganglia (ρ = –.72; P = .005) and in the hippocampus (ρ = –.50; P = .04) (Fig. 1). There were no statistically significant effects of group status or differential group effects in the three brain regions for the other three metabolites measured in this study.

DISCUSSION

Our initial hypothesis, that tamoxifen would increase MI concentrations in the brain, proved incorrect. Instead, we found that women who took tamoxifen or HRT had statistically significantly lower levels of MI in their brains than did women who received no drug therapy. Because normal aging is associated with increases in the cerebral concentrations of MI (24), the reduced MI concentrations that we observed in women who received tamoxifen or HRT suggest that both of these therapies may be associated with the favorable modulation of brain aging.

Table 1. Cerebral metabolite concentrations (mmol/kg)*

Region of the brain/group	Metabolite, mmol/kg			
	NA (95% CI)	CR (95% CI)	CHO (95% CI)	MI (95% CI)†
Basal ganglia				
Tamoxifen	8.51 (8.13 to 8.89)	8.92 (8.30 to 9.54)	2.06 (1.91 to 2.21)	6.33 (5.73 to 6.93)‡
Control	8.66 (8.30 to 9.02)	8.92 (8.54 to 9.30)	2.14 (2.01 to 2.27)	7.57 (6.99 to 8.15)
HRT	8.75 (8.43 to 9.07)	8.97 (8.57 to 9.37)	2.13 (1.98 to 2.28)	6.73 (6.03 to 7.43)
Frontal white matter				
Tamoxifen	7.39 (7.03 to 7.75)	6.69 (6.26 to 7.12)	1.69 (1.58 to 1.80)	7.51 (6.87 to 8.15)
Control	7.42 (7.14 to 7.70)	6.52 (6.22 to 6.82)	1.76 (1.65 to 1.87)	7.57 (7.17 to 7.97)
HRT	7.49 (7.19 to 7.79)	6.37 (6.09 to 6.65)	1.67 (1.54 to 1.80)	7.28 (6.81 to 7.75)
Hippocampus				
Tamoxifen	9.04 (8.53 to 9.55)	8.66 (8.08 to 9.24)	2.80 (2.61 to 2.99)	10.10 (9.42 to 10.78)
Control	8.62 (8.26 to 8.98)	8.32 (7.94 to 8.70)	2.79 (2.64 to 2.94)	10.60 (10.09 to 11.11)
HRT	8.69 (8.24 to 9.14)	8.51 (8.11 to 8.91)	2.74 (2.57 to 2.91)	9.82 (9.29 to 10.35)

*CI = confidence interval; NA = *N*-acetyl-containing compounds; CR = total creatine; CHO = choline-containing compounds; MI = *myo*-inositol; HRT = hormone replacement therapy.

†P = .02 (group effect on analysis of variance).

‡Statistically significantly lower (P≤.004); *t* test, uncorrected for multiple comparisons) than the MI concentration in the basal ganglia of control subjects (P<.04 after correction for multiple [n = 9] comparisons).

The inverse relationship between the MI concentration in the basal ganglia and the length of tamoxifen treatment suggests that longer exposures to tamoxifen may be more beneficial to brain metabolism than short exposures; however, this issue should be addressed more thoroughly in a longitudinal study. The lack of a relationship between the duration of HRT and cerebral metabolite concentrations, however, could suggest that the effects of HRT on brain chemistry may reach a steady state much earlier than 20 years, the average duration of HRT for women in this study.

Results of several recent reports are consistent with our findings and support our conclusion that both tamoxifen and estrogen may have similar effects in the brain. Several preclinical studies (25,26) have suggested that estrogen could be neuroprotective, possibly by blocking oxidative stress-induced neuronal death (27). Another study (28) found that both estrogen and tamoxifen protect glial cells from glutamate-mediated cytotoxicity and stimulate cell differentiation. Furthermore, induction of the expression of aromatase, the enzyme that produces estrogen de novo in astrocytes, is thought to be part of the glial repair response to brain injury (29), and estrogen receptors are expressed in reactive astrocytes in response to injury in the primate brain (30). Finally, both estrogen and tamoxifen increased synaptic density in ovariectomized rats (31). These findings all support a neuroprotective or repair role for estrogen and tamoxifen. Alternatively, based on the observation that glial activity increases with normal brain aging in mice (32), it is possible that the women who received estrogen or tamoxifen had lower concentrations of the glial marker MI in their brains because of a slowing of the aging process in their brains.

Clinical studies provide additional support for our conclusion that estrogen and tamoxifen do not cause brain injury and/or may be neuroprotective. One prospective breast cancer trial (33) separated the effects of tamoxifen and chemotherapy on cognition and found no influence of tamoxifen on patients' self-reports of cognitive function. A large-scale, retrospective cross-sectional study (34) that used the New York State Medical Data System (NYS-MDS) to evaluate 6925 female nursing home residents over 65 years of age found that, compared with women who had never taken tamoxifen, women who took tamoxifen were less likely to have a diagnosis of Alzheimer's disease, were statistically significantly (P<.01) more independent in performing activities of daily living, and had better cognitive skills for daily decision making. Two other studies found no such positive effect of tamoxifen on mental health (35) or cognition (36) among relatively younger women. The first of these studies, from the health-related quality-of-life component of the National Surgical Adjuvant Breast and Bowel Project Prevention Trial, reported baseline and 36-month data for 11 064 women, with an average age of 58 years, that were obtained from two screening questionnaires [Center for Epidemiological Studies-Depression Scale and the Medical Outcomes Study 36-Item Short Form Health Status Survey (35)]. That study found that women treated with tamoxifen had similar depressive symptoms, quality of life, and mental health as did women treated with placebo. By contrast, elderly nursing home residents in the NYS-MDS study who received tamoxifen were 42% more likely to have had a diagnosis of depression than were those who did not receive tamoxifen. In the second study that failed to show a positive effect of tamoxifen on cognition, follow-up questionnaires designed to assess cognitive function (e.g., testing the ability to draw a clock, copy a box, and write a narrative describing a picture) were mailed to 1163 breast cancer patients whose ages ranged from 57 to 75 years. That study (36) found that cognitive function in women who had used tamoxifen for the standard term (4–5 years) was not statistically significantly lower than that in women who had not used tamoxifen, although the women who were current users of tamoxifen complained more about memory problems than the nonusers of tamoxifen. However, these studies had several potential confounding variables, such as the diagnosis of cancer or other chronic medical illnesses, as possible causes for increased depression (30) and the higher frequency of follow-up visits to doctors by current users of tamoxifen than by nonusers (31), which might have accounted for the higher number of memory complaints in the former group. These confounding variables need to be controlled for in future prospective studies.

Taken together, the findings from these published studies are consistent with the normal cognitive screening assessments that we observed for women on tamoxifen in our study. However, these large surveys and our screening assessments of cognition used relatively simple tests of cognitive function, each with recognized sensitivity limitations; therefore, more detailed neuropsychologic tests are necessary to determine whether tamoxifen use is merely nonharmful or whether it has beneficial effects on cognitive function. Future studies should also evaluate the interaction between age and the effects of tamoxifen. Because both studies that found positive effects of tamoxifen on the brain (the NYS-MDS study and our study) included only women aged 65 years or older, it is possible that the apparent neuroprotective effects of tamoxifen are more evident in older patients who might have some decline in cognitive abilities as a result of normal aging.

In contrast to the cognitive assessment surveys, in which responses and/or performance might be confounded by many factors such as effort or depression, our measurements of brain metabolite concentrations by MRS may provide a more objective way to evaluate the integrity of neuronal and glial function. We found no difference in the concentration of NA, which includes the neuronal marker N-acetyl-L-aspartate, between women who received tamoxifen and those who received either HRT or no drug therapy. This result suggests that tamoxifen use is not associated with substantial neuronal injury or loss. In addition, the similar levels of MI in the brains of women who used tamoxifen and those who used estrogen suggest that tamoxifen does not have a negative, antiestrogenic effect on the brain.

Several potential study limitations might affect the interpretation of our findings. First, because of the cross-sectional, nonrandomized design of our study, factors other than drug treatment status might have affected the outcome. For example, differences in brain metabolism among the study groups may be related to vascular abnormalities or motor disorders in these elderly women. However, this possibility is unlikely because we excluded all women with a clinical history of stroke, cortical infarcts on MRI, uncontrolled hypertension, diabetes, or any neuropsychiatric disorders from our study. Furthermore, we conducted two neuropsychologic tests that would have identified those subjects with signs similar to those found in Parkinson's disease (37) and found no differences in test results between the women treated with HRT and those treated with tamoxifen or with no drugs at all. A second limitation of our study concerns

potential differences among the study groups in the amounts of gray and white matter in the MRS volumes of interest, especially in the basal ganglia, which contains mostly deep gray matter with some admixture of white matter. However, a detailed analysis of the measured metabolite concentrations in the various brain regions argues against this possibility. In the control group, the MI concentrations in the basal ganglia and in the white matter were essentially identical, whereas the concentrations of CR and CHO were substantially higher in the basal ganglia than in the white matter. Therefore, a change in the gray/white matter composition of the basal ganglia volume in the tamoxifen or HRT groups relative to the control group would not substantially change the MI concentration but would have a marked effect on the CR and CHO concentrations. The fact that we observed the opposite result—a statistically significant change in MI concentration but not in CR and CHO concentrations—argues that there were no substantial differences in the voxel compositions among the three study groups.

In conclusion, the decreased concentration of the glial marker MI in women taking either tamoxifen or estrogen suggests that both drugs may be neuroprotective and may have favorable modulatory effects on aging. The fact that we detected no evidence of neurotoxicity by ^{1}H MRS, coupled with the accumulating evidence that tamoxifen is either not neurotoxic or even beneficial for cognitive function, further reduces concerns about prescribing tamoxifen to reduce the risk of breast cancer. Future prospective, longitudinal studies involving MRS and detailed neuropsychologic testing of women taking tamoxifen as well as other selective estrogen receptor modulators and aromatase inhibitors that are under evaluation for breast cancer risk reduction are needed to document the long-term effects of such therapies on cognitive function.

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